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Improved cell sensitivity and longevity in a rapid impedance-based toxicity sensor†

Theresa M. Curtis,[a] Joel Tabb,[a] Lori Romeo,[a] Steven J. Schwager,[b] Mark W. Widder[c]* and William H. van der Schalie[c]

ABSTRACT: A number of toxicity sensors for testing field water using a range of eukaryotic cell types have been proposed, but it has been difficult to identify sensors with both appropriate sensitivity to toxicants and the potential for long-term viability. Assessment of bovine pulmonary artery endothelial cell (BPAEC) monolayer electrical impedance with electric cell-substrate impedance sensing (ECIS) showed promise in a previous systematic evaluation of toxicity sensor technologies. The goal of the study reported here was to improve toxicant responsiveness and field portability of this cell-based toxicity sensor. A variety of human cells, non-human mammalian cells, and non-mammalian vertebrate cells were screened for sensitivity to 12 waterborne industrial chemicals. The results of this assessment show that bovine lung microvessel endothelial cell (BLMVEC) monolayers and iguana heart (IgH-2) cell monolayers could detect nine out of the 12 waterborne industrial chemicals, an improvement over the seven chemicals previously detected using BPAEC monolayers. Both the BLMVEC and IgH-2 cell monolayers were tested for their ability for long-term survival on the ECIS test chips in a laboratory environment. Both cell lines were able to maintain high impedance readings on the ECIS electrodes for 37 days, a key trait in developing a field-portable toxicity sensor for water. Cell line optimization has greatly contributed to the on-going development of a field-portable cell-based biosensor that detects with sensitivity a wide range of waterborne toxicants. Published in 2009 by John Wiley & Sons, Ltd.

Keywords: toxicity sensor; electrical cell–substrate impedance sensing; ECIS; cell monolayers

Introduction

Options for rapid analysis of chemical contaminants in water are limited, but more thorough analysis for a broad range of organic and inorganic chemicals requires complex instrumentation not readily available in field situations. One alternative is to use sensors that rapidly evaluate the toxicity of a whole water sample instead of measuring concentrations of individual chemical constituents. Biologically based toxicity sensors can provide rapid assessments of water quality and can contribute to drinking water security investigations (States *et al*., 2004). Sensors that evaluate cellular cytotoxic responses are well-suited for a broad range of sensing applications, including the detection of unknown agents, and the use of mammalian cells as biosensors can provide responses relevant to human physiology (Pancrazio *et al*., 1999; Kovacs, 2003). A wide range of cellular responses have been proposed for use in cell-based biosensors (Ziegler, 2000), including the measurement of the electrical impedance of cell monolayers (Giaver and Keese, 1992; Keese *et al*., 1998).

One device that measures changes in cellular impedance is the electric cell–substrate impedance sensing (ECIS) system (Giaever and Keese, 1993), which can provide quantitative information on cell morphological changes under various chemical and biological treatments (Giaever and Keese, 1986, 1991; Tiruppathi *et al*., 1992; Garcia *et al*., 1997; Keese *et al*., 1998; Luong *et al*., 2001; Xiao *et al*., 2002). The ECIS system has been used specifically to assess the cytotoxicity of a variety of toxicants. Most of the toxicants tested in the ECIS system used a cytotoxicity protocol that treated cells in suspension with the toxicant, and then monitored their ability to adhere to the ECIS electrodes over several hours (Luong *et al*., 2001; Xiao *et al*., 2002; Xiao and Luong, 2005). This method is not adaptable for field water testing because too many steps are required and the assay takes too long (up to 24 h) to complete. An alternative protocol measures the impedance of established cell monolayers after toxicant addition (Arndt *et al*., 2004; Giaver and Keese, 1992; Keese *et al*., 1998). This simpler method has the potential for translation into a field-portable assay, but has only been validated with a few toxicants.

In an evaluation of toxicity sensors for rapid testing of drinking water, the ECIS system (in a 1 h assay format using confluent bovine pulmonary artery endothelial cell (BPAEC) monolayers) was compared with nine other toxicity sensors in a blind study using 12 chemical toxicants (van der Schalie *et al*., 2006). The ECIS-based sensor was one of three sensors that responded to the most chemicals in the desired sensitivity range between the Military Exposure Guidelines (MEG) concentration (a threshold level believed to pose minimal to no human health threat; USACHPPM, 2004) and the estimated Human Lethal Concentration (HLC; TERA,

* Correspondence to: M. W. Widder, US Army Center for Environmental Health Research, 568 Doughten Drive, Fort Detrick, MD 21702-5010, USA. E-mail: mark.widder@us.army.mil

a Agave BioSystems, Inc., 401 E. State Street, Ithaca, NY 14850, USA.

b Cornell University, Department of Biological Statistics and Computational Biology, 1194 Comstock Hall, Ithaca, NY 14853, USA.

c US Army Center for Environmental Health Research, 568 Doughten Drive, Fort Detrick, MD 21702-5010, USA.

** Present address: Department of Biological Sciences, State University of New York at Cortland, Cortland NY 13045, USA.

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14. ABSTRACT

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2006). The ECIS-based sensor also showed very low reactivity to potential interferences such as hard water and common water additives, making it an attractive system for water monitoring applications. Because the ECIS sensor using BPAEC monolayers detected seven of 12 chemicals in the target concentration range within an hour, this sensor shows great promise for field water testing.

Thus, the primary goal of this study was to improve the toxicant responsiveness of the ECIS-based sensor by selecting the most sensitive of a variety of candidate cell lines, using the same chemicals tested by van der Schalie *et al.* (2006). Ten cell lines were selected for evaluation including human, non-human mammalian and non-mammalian vertebrate cells. Selected liver, kidney, skin, intestinal and vascular cells offered the possibility of toxicant sensitivity not found in other cell types. The non-mammalian vertebrate cells had the advantages of not requiring carbon dioxide for culturing and potentially greater tolerance of temperature variations than the mammalian cells, which could improve their suitability for a field-portable toxicity sensor system. Human cell responses appear to be more predictive of lethal toxicant concentrations in human blood than non-human cells (Ekwall *et al.*, 1998).

One major issue with using mammalian cells in toxicity sensors is the difficulty of maintaining cell viability under field conditions for extended periods of time and with minimal support until they are needed for water testing (Rudolph and Reasor, 2001). This was a problem encountered in previously developed portable toxicity sensors using cardiomyocytes (DeBusschere and Kovacs, 2001) and neurons (Pancrazio *et al.*, 2003, 2004). In addition to improving toxicant sensitivity, an additional study goal was to determine the viability of selected cell lines in the ECIS system with minimal maintenance, because the length of time that cells can be held prior to testing is an important issue in the development of a field-portable system. The results of this study show that the bovine lung microvessel endothelial cell (BLMVEC) monolayers and the iguana heart cell (IgH-2) monolayers can improve both the sensitivity of response to toxicants and the longevity of cells in the ECIS system as compared with the currently used BPAEC monolayers.

Materials and Methods

Tissue Culture and Cell Seeding on Electrodes

Ten cell lines were selected for comparison to the BPAEC monolayers used in the initial toxicity evaluation (van der Schalie *et al.*, 2006). Table 1 lists the cell lines and culture methods used to determine whether the cell lines could form confluent monolayers on the ECIS chips (Applied BioPhysics, Troy, NY, USA) and provide

Table 1. Culture conditions for the cell lines tested for suitability in the ECIS system

Cell line	Source[a]	Media[b]	Adhesion substrates[c]	Temperature (°C)	CO_2
Bovine pulmonary artery endothelial cells (BPAEC)	VEC Technologies (Rensselaer, NY)	α-Minimal essential medium (α-MEM) and 20% fetal bovine serum (FBS)	Gelatin	37	5%
Bovine lung microvessel endothelial cells (BLMVEC)	VEC Technologies	MCDB-131C (VEC Technologies)	Fibronectin or gelatin	37	5%
Human intestinal epithelial cells (Caco2; C2BBe1)	HTB-37; CRL-2102	Dulbecco's modified Eagle's medium (DMEM)[d] and 10% FBS	Collagen I or collagen IV	37	5%
Fish liver epithelial cells	CRL-2406	Eagle's minimum essential medium (EMEM)[e] and 5% FBS	Collagen I, laminin or uncoated	30	5%[f]
Human liver epithelial cells (Hep-G2)	HB-8065	EMEM[e] with 10% FBS	Fibronectin, laminin or gelatin	37	5%
Human umbilical vein endothelial cells (HUVEC)	VEC Technologies	MCDB-131C (VEC Technologies)	Fibronectin or gelatin	37	5%
Iguana heart cells (IgH-2)	CCL-108	EMEM with Hanks balanced salt solution (HBSS)[g] and 10% FBS	Fibronectin, laminin or gelatin	37	No
Human keratinocytes	Cambrex Bio Science (Walkersville, MD); CC-2507	KGM-2 (Cambrex Bio Science)	Fibronectin, laminin or poly-lysine	37	5%
Turtle heart cells (TH-1)	CCL-50	Basal medium (Eagle) with HBSS and 10% FBS	Fibronectin, laminin or gelatin	25	No
African green monkey kidney cells (Vero cells)	CCL-81	EMEM[e] with 10% FBS	Fibronectin, laminin or gelatin	37	5%

[a]All cells purchased from American Type Culture Collection (ATCC; Manassas, VA, USA) unless otherwise noted.
[b]All media purchased from Invitrogen (Carlsbad, CA, USA) unless otherwise noted.
[c]All adhesion substrates purchased from Sigma-Aldrich (St Louis, MO, USA) except fibronectin, collagen I and IV, which were purchased from Calbiochem (San Diego, CA, USA).
[d]Plus 4 mM L-glutamine, 4.5 g l^{-1} glucose, 1.5 g l^{-1} sodium bicarbonate, 0.01 mg ml^{-1} human transferrin.
[e]Plus 2 mM L-glutamine and Earle's BSS adjusted to contain 1.5 g l^{-1} sodium bicarbonate, 0.1 mM non-essential amino acids and 1.0 mM sodium pyruvate.
[f]Can be adapted for CO_2-independent growth.
[g]Plus 2 mM glutamine and 0.1 mM non-essential amino acids.

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stable impedance readings over a two-week storage time. Except for the standard BPAECs, each cell line was evaluated with at least two adhesion substrates (Table 1) to increase the likelihood that a stable and uniform monolayer could be formed on the gold electrodes. Adhesion substrates were prepared according to manufacturers' instructions, and 200 μl of each diluted substrate [laminin (25 μg ml^{-1}); fibronectin (50 μg ml^{-1}); collagen I and IV (25 μg ml^{-1}); poly-D-lysine (100 μg ml^{-1}); and gelatin (0.2% solution)] was added to each well, incubated for 1–2 h, and washed with growing medium before use. Each cell line was seeded over a range of cell densities (0.4–1.5 × 10^5 cells/well), maintained by periodic feeding, and stored for a two-week period during which the impedance was recorded as an indication of the monolayer integrity and general cell health. A cell monolayer was determined to be stable over the two-week storage period if the impedance values did not decrease more than 20%, and if the morphology of the cells did not change substantially as visualized by phase contrast microscopy. The sensitivity of the system depends on the presence of a confluent cell monolayer that can effectively impede ion flow. The 20% reduction in impedance was defined as an acceptable reduction based on previous experiments using confluent cell monolayers for toxicity testing (van der Schalie *et al*., 2006).

Cell lines found suitable for use in the ECIS system were further tested for their ability for long-term survival on the ECIS test chips. Cell lines were seeded onto ECIS electrode arrays (three to four replicates) and stored for 37 days. During this maintenance time, the cell monolayers were fed three times a week by medium renewal. Different adhesion substrates and fetal bovine serum concentrations were tested to optimize the maintenance of the cell layers over time. Impedance levels were measured periodically over the 37 day period. The percentage reduction in impedance over time was calculated. Stable monolayer maintenance over the 37 day period is defined as less than a 20% reduction in impedance values over time.

Impedance Measurement with ECIS

Impedance of cell monolayers grown on ECIS chips was monitored using the ECIS 1600 analyzer (Applied BioPhysics, Troy, NY, USA) as described in Luong (2003). Briefly, the cells were seeded on ECIS chips (8W10E; Applied BioPhysics, Troy, NY, USA) and grown to confluence. On the bottom of each well were 10 small working gold electrodes (each electrode is 250 μm in diameter) and one large counter gold electrode. A 15 000 Hz signal with 1 V amplitude was applied to the cells through a 1 MΩ resistor, creating a current source. Current flowed between the small working electrodes and the counter electrode through the cell culture medium that bathed the electrodes and served as the electrolyte. The voltage between the small and large electrode was monitored by the lock-in amplifier, stored and processed by a personal computer. Because the surface area ratio between the working electrodes and the counter electrodes was small, the impedance of the working electrode interface dominated the value.

When cells attach and spread, they form a confluent monolayer on the working gold electrode, which acts as an insulating layer because the plasma membrane interferes with current flow above the electrode. Consequently, there is a drastic increase in impedance when the cells form a confluent cell barrier. When cell monolayers grown on the electrode undergo any change in cell–cell interaction or cell–substrate interaction, there are readily measurable changes in impedance (Giaever and Keese, 1993).

Chemicals Used in Toxicity Testing

Chemicals used for toxicity testing (Table 5) were obtained from Fisher (Fairlawn, NJ, USA) or ChemService (West Chester, PA, USA) and were selected to represent different chemical classes and modes of toxic action. All stock solutions were prepared in deionized water at the US Army Center for Environmental Health Research (Ft Detrick, MD, USA). The ECIS-based sensor using BPAECs previously showed very low reactivity to potential interferences such as hard water and common water additives, so the chemicals in the current study were prepared in deionized water (van der Schalie *et al*., 2006). The only exception was pentachlorophenol, which was prepared from the sodium salt of pentachlorophenol in 2 mM phosphate buffer and pH adjusted to 7.5. Stock solutions were used in testing within two weeks, except for toluene and nicotine, which were used immediately after preparation due to volatility and stability issues, respectively.

To prepare the chemical toxicants for cell testing, twice-concentrated serum-free culture medium (with 0.5% bovine serum albumin, BSA) was diluted 50:50 with the test chemical solutions to ensure correct osmolarity of the samples prior to addition to the cell monolayers. Each test solution was brought to the appropriate test temperature (Table 1) by holding samples in an incubator for 30 min prior to testing.

Toxicity Testing Procedures

Screening toxicity tests were performed for each cell line found suitable for use in the ECIS system. Aldicarb and sodium pentachlorophenate were selected for screening because the standard BPAEC monolayers have been found to be relatively insensitive to these chemicals in previous toxicity testing (van der Schalie *et al*., 2006). For the screening toxicity tests, there were two control wells and three sets of replicate toxicant concentrations on each ECIS chip. Two chips were used per chemical, providing four control wells plus four toxicant-exposed wells at each of three toxicant concentrations.

Cell lines showing the greatest improvement over the standard BPAEC monolayers in sensitivity in the screening tests were tested with the full set of 12 chemicals. In these tests, each ECIS chip had two controls and three sets of replicate toxicant concentrations. Two to six chips were used per chemical, providing four to 12 replicates of control wells and each of the three toxicant concentrations.

To acclimate cells to the test conditions, growth medium containing serum was removed from the cell monolayers and was replaced with serum-free medium (with 0.5% BSA) 1 h prior to testing. After 1 h, impedance values were measured and recorded for each well prior to chemical addition, to serve as a baseline measurement. The medium was then removed and either 400 μL of the prepared test chemical solution or 400 μL of serum-free medium (control wells) was added to the wells. Impedance values were monitored in each well every minute for approximately 60 min. Tests were conducted in a cell culture incubator at temperature and CO_2 levels appropriate for each cell line.

The impedance readings were normalized by dividing the impedance measured at each time point by the initial impedance value (prior to chemical exposure) in each well. This was necessary because the starting impedance value in each well was slightly different due to the variability of the cells covering the

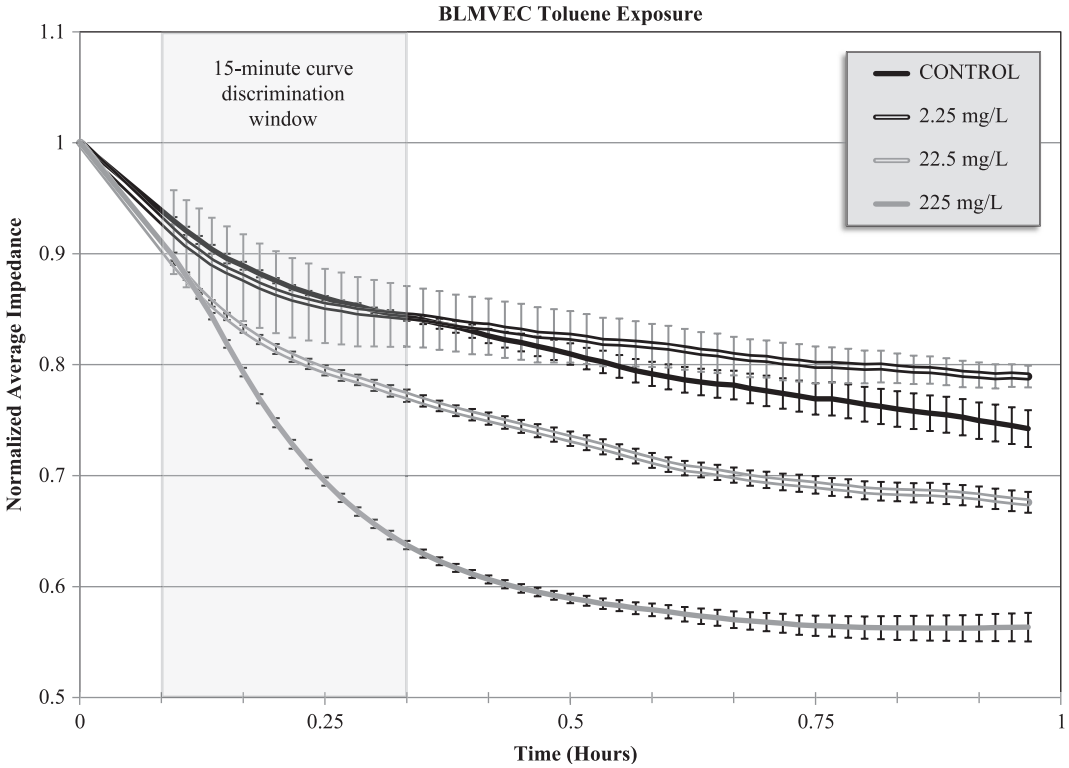

Figure 1. Time course of normalized average impedance response curve of various concentrations of toluene in relationship to centrals. The 15-minute window for curve discrimination evaluation is highlighted. ($n = 6$ per treatment)

electrodes. Normalizing the data allowed for a more consistent comparison of observed impedance differences.

To determine when the response curves generated by toxicant-exposed cells differed significantly from the curves generated by the control, a curve discrimination program was written using MATLAB (The MathWorks Inc., Natick, MA, USA). This program analyzed impedance data from control and toxicant-exposed cells during a 15 min window that was chosen from the 60 min of data based on observation. The overall difference between the two groups of curves was measured at each time point, and the difference between their averages was assessed relative to the within-group variability. A confidence level of 95% ($P < 0.05$) was used to establish statistical significance. The lowest toxicant concentration that caused a significant change in impedance was reported as the lowest effect concentration in Tables 3 and 5. Functional data analysis techniques (Ramsay and Silverman, 2005) were used to extend the standard analysis of variance approach for a single time point (Ott and Longnecker, 2000) to analysis of a curve consisting of approximately 60 points. An example of an impedance response curve of control vs toxicant-treated cells is provided in Fig. 1. Figure 1 also depicts the 15 min window for curve discrimination analysis. The time period from 5 to 20 min was found to provide the most rapid response time while avoiding false positive detects of non-toxicant water samples.

Results and Discussion

Suitability of Alternative Cell Lines for Use in the ECIS System

To screen chemical cytotoxicity within 1 h in a field environment, the cell monolayers chosen must have sensitivity to a variety of toxicants, stability over long periods of time, and simple culturing requirements that allow reproducible results to be obtained. Based on these criteria, various endothelial and epithelial cell lines were screened for use in the ECIS system because of their ability to form a tight diffusion barrier that is stable over extended periods of time. Table 2 summarizes the performance of cell lines in preliminary testing in the ECIS system. Some of the cell lines, such as the fish and human liver cells, were unable to form a cell layer on the ECIS electrodes that would remain stable for two weeks. Human keratinocytes formed a cell monolayer, but the requirements for daily care made these cells impractical for use in a toxicity sensor system ultimately intended for field use. The human intestinal epithelial cells initially displayed impedances over 1000 Ω, but over a two-week storage time, the impedances decreased to 800–900 Ω, which correlated with an increase in the formation of fluid-filled domes. These domes form when Caco-2 cells are grown on non-porous surfaces; they are thought to result from the transport of fluid across the cell layer (Lopez-Vancell *et al*., 1984) and may be responsible for the decrease in impedance over time. The turtle heart cells formed a monolayer, but impedance values were too low, indicating that they would not be sensitive to chemical toxicity because of the limited range of impedance between the healthy and dead cells. The remaining four cell lines (HUVECs, Vero cells, IgH-2 cells and BLMVECs) formed stable monolayers on the ECIS electrodes, and were screened for toxicant sensitivity.

Chemical Sensitivity Using Alternative Cell Types

The four cell lines found suitable for use with the ECIS system were evaluated for their sensitivity to aldicarb and pentachlorophenate (Table 3) relative to the standard BPAECs. The HUVECs

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Table 2. Suitability of cell lines for use in the ECIS system: 'X' = unsuitable; '√' = acceptable

Cell line	Formation of stable cell monolayer[a]	Ease of maintenance	High impedance (> 1000 Ω)	Preliminary toxicant screening
Fish liver epithelial cells	X			
Human liver epithelial cells (Hep-G2)	X			
Human keratinocytes	√	X		
Human intestinal cells (Caco-2, C2BBe1)	√	√	X	
Turtle heart cells (TH-1)	√	√	X	
Human umbilical vein endothelial cells (HUVECs)	√	√	√	√
African green monkey kidney cells (Vero cells)	√	√	√	√
Iguana heart cells (IgH-2)	√	√	√	√
Bovine lung microvessel endothelial cells (BLMVECs)	√	√	√	√

[a]Formation of stable monolayer in this report is defined as less than a 20% reduction in impedance values over the two-week storage time.

Table 3. Toxicant sensitivity of five cell monolayers to both aldicarb and pentachlorophenate in the ECIS system

	Lowest effect concentration (μM)				
Toxicant	BPAECs[a]	BLMVECs	IgH-2 cells	HUVECs	Vero cells
Aldicarb	1270	105	105	1050	>1050
Pentachlorophenate	20	3.8	38	380	380

[a]Data from van der Schalie *et al*. (2006).

Table 4. Long-term maintenance of BPAEC, BLMVEC, and IgH-2 cell monolayers using different protein matrices and fetal bovine serum levels

Cell type	Adhesion substrate	Fetal bovine serum levels	Impedance values (Ω) Mean ± SD (n = 3–4)			Percentage reduction in impedance over storage time
			Day 5	Day 17	Day 37	
BPAEC	Gelatin	1%	771 ± 20	577 ± 132	681 ± 346	12%
		20%	780 ± 27	677 ± 76	656 ± 105	16%
	Fibronectin	1%	850 ± 24	713 ± 62	673 ± 163	21%
		20%	570 ± 157	868 ± 102	679 ± 101	—
BLMVEC	Gelatin	1%	866 ± 241	1120 ± 202	1836 ± 284	—
		10%	1562 ± 46	1833 ± 58	1967 ± 52	—
	Fibronectin	1%	1158 ± 482	2087 ± 134	2218 ± 33	—
		10%	2064 ± 87	2042 ± 65	2004 ± 26	3%
IgH-2						
	Gelatin	1%	1444 ± 77	1660 ± 82	1333 ± 101	8%
		10%	1648 ± 107	1660 ± 47	1356 ± 91	18%

and Vero cells were far less sensitive to pentachlorophenate and comparably sensitive to aldicarb, while the BLMVECs and IgH-2 cells were comparably sensitive to pentachlorophenate and much more sensitive than the BPAECs to aldicarb. Based on this initial evaluation, the IgH-2 cells and the BLMVECs were chosen for a complete evaluation against the panel of 12 chemicals.

As shown in Table 5, the BLMVEC and IgH-2 cell monolayers were more sensitive than the BPAEC monolayers to all chemicals tested. The only exceptions were BLMVECs tested with arsenic and IgH-2 cells tested with pentachlorophenate and toluene. The greatest increase in sensitivity, nearly two orders of magnitude, was for nicotine. Although ammonia and cyanide were detected below the 7–14 day MEG concentration, the detection limit was above the one-year MEG in both cases (USACHPPM, 2004), indicating that a response at these levels has human health significance. Including responses between the one-year and 7–14 day MEGs as appropriate, the BPAEC monolayers responded to seven chemicals within the desired sensitivity range, while the

Table 5. Toxicant sensitivity of BLMVEC and IgH-2 cell monolayers compared with BPAEC monolayers in the ECIS system. Chemicals used in toxicity tests with the response range in comparison to the 7–14 day Military Exposure Guidelines (MEG) and the Human Lethal Concentration (HLC)

Toxicant	Chemical Abstract Service (CAS) number	Desired sensitivity range		Lowest effect concentration (μM) $n = 4$[d]		
		MEG[a] (μM)	HLC[b] (μM)	BPAEC[c]	BLMVEC	IgH-2
Aldicarb	116-06-03	0.026	0.89	1270	105	105
Ammonia	7664-41-7	1761	54 300	1800	400	400
Arsenic	7758-98-7	0.267	60	10	40	4
Copper	7487-94-7	2.20	1620	110	8	8
Cyanide	10265-92-6	76.9	540	800	230	23
Mercury	54-11-5	0.005	120	30	2.5	2.5
Methamidophos	4685-14-7	0.014	10	630	>1170	117
Nicotine	108-95-2	0.801	100	3900	40	40
Paraquat	7784-46-5	0.194	18	1940	97.2	972
Pentachlorophenate	143-33-9	0.526	270	20	3.8	38
Phenol	131-52-2	31.9	970	780	270	270
Toluene	108-88-3	10.9	9120	800	240	2400

[a]USACHPPM (2004). Concentrations (μM converted from mg l^{-1}) are for 7–14 day consumption, 15 l day^{-1}, except for copper (1 year MEG) and nicotine (<7 day MEG).
[b]TERA, 2006, assuming a 70 kg person consuming 15 l day^{-1}. These values (μM converted from mg kg^{-1}) have been revised from those used in van der Schalie *et al*. (2006).
[c]Data from van der Schalie *et al*. (2006).
[d]Four replicates were used per treatment for all tests except $n = 8$ for copper and mercury exposure to IgH-2, and $n = 12$ for arsenic exposure to IgH-2.

BLMVEC and IgH-2 cell monolayers each responded to nine chemicals. Based on toxicant response characteristics, both the BLMVEC and IgH-2 cell monolayers are an improvement over the standard BPAEC monolayers previously used in the ECIS based sensor.

Stability of Monolayers Over Extended Storage Times

To create a cell-based toxicity sensor suitable for field use, the cell monolayers must be stable on the ECIS electrode arrays during what may be an extended period of storage before introduction of a water sample to the test system. As shown in Table 4, BPAEC, BLMVEC and IgH-2 cell monolayers could be stored for 37 days on the ECIS electrode arrays with minimal change in impedance. However, the low initial impedance values for the BPAEC cells suggests that the BLMVECs and IgH-2 cells may have better dynamic responses in the event of toxicant exposure. Further, it appears that 1% serum was sufficient for long-term maintenance of the cells, but the use of serum-free medium caused all three cell lines to die within a week (data not shown). There was no apparent effect associated with the use of different adhesion substrates. The significant increase in impedance observed in the BLMVEC monolayers over time was not the result of cells layering on top of the existing monolayer, as observed by phase contrast microscopy (data not shown). The increase in impedance was most likely caused by a change and/or maturation in cell–matrix or cell–cell junctions over time.

Conclusions

A number of toxicity sensors for water using a range of eukaryotic cell types have been proposed; however, it has been difficult to identify sensors that have both the appropriate sensitivity to toxicants and the potential for long-term viability that might provide the basis for a toxicity sensor suitable for real-world applications. Using ECIS technology to measure the electrical resistance of endothelial cell monolayers showed promise in a previous systematic evaluation of toxicity sensor technology. The current study showed that four of 10 cell lines were suitable for use in the ECIS system and two of these (BLMVEC and IgH-2 cells) showed improved sensitivity to toxicants over the previously used BPAEC monolayers. In addition, the BLMVEC and IgH-2 cell monolayers were able to maintain high impedance readings on the ECIS electrodes for at least 37 days, a key requirement for developing a field-portable toxicity sensor for water.

To improve the suitability of ECIS technology for field use, an enclosed fluidic biochip and automated instrument that can both support the health of the cell monolayers over time and take impedance measurements are being tested. Adding an automated sample injection system will further reduce the need for user interaction, and the anticipated decrease in chip response variability associated with automation should increase the detection sensitivity of the system.

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